DR ANTONY RICHARD YOUNG (Orcid ID : 0000-0002-4163-6772)

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Children sustain high levels of skin DNA photodamage, with a modest increase of serum 25(OH)D_3, after a summer holiday in Northern Europe

J. Narbutt[1], P.A. Philipsen[2*], A. Lesiak[1], T. Sandberg Liljendahl[4], D. Segerbäck[4], J. Heydenreich[2], D. Chlebna-Sokol[5], P. Olsen[2], G.I. Harrison[6], A. Pearson[7], K. Baczynska[7], M. Rogowski-Tylman[3], H.C. Wulf[2], A.R. Young[6]

[1]Medical University of Łódź, Second Department of Dermatology, Dermatology, Paediatric and Oncology Clinic, 91-347 Łódź, Poland, [2]University of Copenhagen, Bispebjerg Hospital, Department of Dermatological Research, Bispebjerg Bakke 23, Copenhagen 2400, Denmark; [3]Medical University of Łódź, Department of Dermatology, 90- Łódź, Poland; [4]Karolinska Institute, Department of Biosciences and Nutrition, Novum S-141 83, Huddinge, Sweden; [5]Department of Paediatric Propedeutics and Bone Metabolic Diseases, Medical University of Łódź, 90-419 Łódź, Poland; [6]King's College London, St John's Institute of Dermatology, London, SE1 9RT, UK; [7]Public Health England, Radiation Dosimetry, Centre for Radiation, Chemical and Environment Hazards, Chilton, Didcot, Oxon, OX11 ORQ, UK

*** Equal first author**

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Corresponding author:

Antony R Young

antony.young@kcl.ac.uk

St John's Institute of Dermatology, King's College London, Guy's Hospital, London SE1 9RT, UK

Tel: 44 (0)20 7188 6367

Running Head: Impact of summer sun on children's health

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Conflict of interest:

The authors state no conflicts of interest

What's already known about this topic?

- Tenerife holiday studies in adults have shown a UVB dependent increase in 25(OH)D_3 and potentially mutagenic cyclobutane pyrimidine dimers (CPD) that may initiate skin cancer.
- Childhood solar UVR exposure increases the risk of skin cancer in adulthood, but no study has assessed the risks and benefits of solar UVR in children on holiday.

What does this study add?

- Relatively low daily UVR doses in children at a Baltic Sea summer camp resulted in a modest but significant improvement of 25(OH)D_3 (24%) but a very much greater increase in CPD (1262%).
- The children had the same level of CPD as adults who had higher UVR doses over a shorter holiday in Tenerife.
- These results stress the importance of rigorous photoprotection in the young.

Abbreviations: BSA: Body surface area, CPD: Cyclobutane pyrimidine dimer, SED: Standard erythema dose, SPF: Sun protection factor, T<>T: thymine dimer

Abstract

Background: Childhood solar ultraviolet radiation (UVR) exposure increases the risk of skin cancer in adulthood, which is associated with mutations caused by UVR-induced cyclobutane pyrimidine dimers (CPD). Solar UVR is also the main source of vitamin D, essential for healthy bone development in children.

Objectives: The impact of a 12-day Baltic Sea (54^{o}N) beach holiday on serum 25-dihydroxyvitamin D ($25(OH)D_3$) and CPD was assessed in 32 healthy Polish children (skin types I-IV).

Methods: Blood and urine were collected, before and after the holiday, and assessed for $25(OH)D_3$ and excreted CPD respectively, and personal UVR exposure was measured. Diaries were used to record sunbathing, sunburn and sunscreen use. Pre- and post holiday skin redness and pigmentation were measured by reflectance spectroscopy.

Results: The average daily exposure UVR dose was 2.4±1.5 (SD) standard erythema doses (SED) which is borderline erythemal. The mean concentration of $25(OH)D_3$ increased (x1.24±0.19) from 64.7±13.3 $\rightarrow$ 79.3±18.7 nmol/L ($p=1.59x10^{-7}$). Mean CPD increased 12.62±10.0-fold from 26.9±17.9 to 248.9±113.4 fmol/μmol creatinine ($p=2.66x10^{-11}$). Increased $25(OH)D_3$ was accompanied by a very much greater increase in DNA damage associated with carcinogenic potential. Overall, skin type had no significant effects on behavioural, clinical or analytical outcomes, but skin types I/II had more CPD (unadjusted p=0.0496) than skin types III/IV at the end of the holiday.

Conclusions: Careful consideration must be given to health outcomes of childhood solar exposure, and a much better understanding of the risk/benefit relationships of such exposure is required. Rigorous photoprotection is necessary for children, even in Northern Europe.

Key Words: Children, UVR exposure, vitamin D, DNA damage

Introduction

The growing global incidence of skin cancer is attributed to increased exposure to solar ultraviolet radiation (UVR). One study predicted that regular use of a sun protection factor (SPF) 15 sunscreen for the first 18 years would reduce the lifetime incidence of keratinocyte cancers (KC) by 78% [1], assuming accumulation of ~50% of lifetime UVR exposure in that time [2]. In contrast, measured solar UVR exposure in Danish children and teenagers suggested that only 25% of lifetime UVR exposure is obtained by 20 years [3]. Thus, measurements of childhood UVR exposure are important because this is a risk factor for malignant melanoma (MM) and basal cell carcinoma (BCC) in adulthood [4-6]. Furthermore, the incidence of childhood and adolescent MM is increasing in the US [7], and the incidence of BCC is increasing in those under 30 years in the UK [8].

Solar UVB (~295-320nm) is the main source of vitamin D [9,10]. The most widely used indicator of vitamin D status is serum 25(OH)D. Vitamin D insufficiency (25(OH)D < 50 nmol/L), or deficiency (25(OH)D < 25nmol/L), prevalent in children [10-12], impairs bone mineralization. A UK population based study of 1102 children (4-18 years) showed that insufficiency was 35% overall and 85% in those that were "non-white" [11]. Rickets is common in the developing world [13], and hospitalisation of children <15 years for this disease in England is at its highest rate for five decades, and not confined to those with pigmented skin [14]. Sub-optimal serum 25(OH)D in childhood may also have adverse effects on tuberculosis and asthma [10,15]. However, there is considerable controversy about the benefits of vitamin D to non-skeletal health [16].

The spectral region that initiates the synthesis of vitamin D, via the conversion of cutaneous 7-dehydrocholesterol to pre-vitamin D, is also the main cause of sunburn (erythema) and epidermal DNA damage [17], especially the formation of cyclobutane pyrimidine dimers (CPD) that are associated with mutations that initiate KC [18]. CPD may also initiate photoimmunosuppression [19], which has been implicated in KC and MM [20]. DNA damage in childhood may be a critical event for adult skin cancer risk. Most studies have made CPD assessments from skin biopsies but it is possible to measure CPD in urine because of their excretion after nucleotide excision repair (NER). This non-invasive approach has been validated [21,22] and has ethical advantages. To date, only one study has assessed the urinary excretion of CPD in children after two consecutive days on a Swedish beach in late summer, and reported a linear increase with UVR dose over an estimated ~70% of body surface area (BSA) exposed [23].

Laboratory studies in adults have shown that epidermal DNA damage accumulates [24] and vitamin D synthesis [25,26] occurs with repeated sub-erythemal UVR exposure. However, we lack data on molecular biomarkers of risk and benefit from "real life" UVR exposure in children. We report on changes in such biomarkers in children after a summer camp holiday. The primary aim was to determine the benefit of holiday solar UVR exposure. This was assessed by measuring serum 25(OH)D_3 along with parathyroid hormone (PTH) and bone turnover markers [27]. The secondary aim was to assess the harmful effects, including erythema, pigmentation (a response to DNA damage) and urinary CPD. In addition, cumulative personal exposure to UVR was measured to determine any possible dose response relationships with these outcomes and, for CPD, also within sub-periods of UVR exposures because induction and repair kinetics of CPD are unknown in children. Additional aims were to (i) determine sun exposure behaviour by the use of daily diaries and any influence of skin type and gender

on all outcomes (ii), determine 25(OH)D_3 in the Autumn, more than 3 months after the holiday and (iii) add to the literature on the proportion of ambient UVR exposure received by children.

Materials and methods

The Medical University of Łódź Bioethics Committee, Poland approved the study, which was done according to the "Declaration of Helsinki". Thirty-two healthy children (girls 22; boys 10; mean age 8.9 years, range 8-10 years with Fitzpatrick [28] skin types I/II (n = 18) and III/IV (n = 14) were selected after a parents' meeting. Exclusion criteria included regular oral or topical medication and sunny holidays within the previous two months. Two parents or a single parent, according to family circumstances, gave informed written consent. The children underwent a thorough paediatric assessment, which was normal in all cases.

The holiday (23rd June-6th July 2009) started the day after the end of the school year. The parents supplied sunscreens. The location was a Baltic Sea (Sztutowo 54^{o}N) camp with an East–West unshaded sandy beach. Blood and urine samples were collected 24h before and 24h after the holiday, and stored at -20^{o}C. Blood samples were also taken from 29/32 participants on 19-20 October 2009 for a follow-up assessment of 25(OH)D_3.

Diaries and measurement of UVR exposure

Each child completed a daily diary, based on previous studies[29,30], of which some parameters are listed in the first two columns of table 1, and wore a personal dosimeter "SunSaver" [31] on the wrist during the day. The dosimeter readout was the standard erythema dose (SED) integrated and reported every 0.5h. Two SunSavers, in watertight housing, on a 2m pole in an open unshaded area monitored ambient UVR.

Skin reflectance

Skin reflectance was measured, 24h before and 24h after the holiday, on 6 sites (back of hand, forehead, upper inner and outer arm, back and upper buttock) (UV-Opimize, Chromo-light, Espergærde, Denmark) at 555nm (erythema) and at 660nm (pigmentation).

Blood parameters

Measurement of 25(OH)D_3

The LC-MS/MS techniques have been described [32]. Samples were run in triplicate, the mean value of which was used as the endpoint. Pre- and post-exposure samples from the same child were always run concurrently.

Serum PTH, crosslaps and osteocalcine

PTH was assessed by immunochemiluminescence (IMMULITE Turbo intact PTH, Diagnostic Products Corporation, Los Angeles USA) and ELISA was used for serum crosslaps and osteocalcine (bone gla protein (BGP) (ELISA Immunodiagnostic Systems GmbH (IDS GmbH), Frankfurt am Main, Germany). The mean of duplicates was used in the analyses.

Urinary CPD

Urine aliquots of 10 µL were analysed for thymine dimers (T<>T), the most frequent CPD, by a postlabelling HPLC method [21]. Creatinine concentration was used to adjust for urine dilution, using the Jaffe method [33].

Statistics

No holiday or laboratory UVR-intervention studies had been previously done in children. The study was overpowered based on a laboratory UVB intervention study of 50 adults[34].

Under this study's conditions, 16 children would have been sufficient to give a significance level of 5% and 90% power to detect Δ23.3 nmol/L 25(OH)D_3 with a SD of 26.5 using a paired design. Analyses were performed using SPSS Statistics version 22 (IBM New York, NY). All data were normally distributed (Kolmogorow-Smirmnov test $p>0.134$), except for the number of sunburns on the individual body sites listed in table 1. All analyses were performed with parametric tests, except for the influence of skin type on the number of sunburns for which the Mann-Witney U-test was used.

Comparisons of pre- and post-holiday values of 25(OH)D, T<>T, PTH, crosslaps, osteocalcin and skin reflectance measurements were made using paired t-tests assuming equal variance, because Levene's (F test for equal variance) tests were non-significant in all cases. The influence of gender, skin type and low/high UVR exposure on numeric measurements, such as 25(OH)D, T<>T and their delta values (post-holiday minus pre-holiday), were made using independent t-tests.

The Chi-squared test was used to test for skin type and gender influence on the categorical diary data outcomes that are summarised in table 1. Pearson correlation was calculated to investigate relationships between individual measured parameters of UVR dose, 25(OH)D, T<>T, PTH, crosslaps, osteocalcin, skin reflectance and measurements at June, July and October time points and their Δ values. Analyses were also done with the temporally sub-divided UVR-dose to determine if any relationships with T<>T were time-dependent. Only relevant correlations were performed and reported, an example of non-relevant correlation is between June PTH and July crosslaps.

General linear models (GLM), which correspond to multiple linear regression analysis, were used to determine the relationships between July and October values for 25(OH)D and their June (baseline) values, controlling for gender and/or skin type. A similar approach was used for T<>T except the baseline was not incorporated, because it would have been cleared during the holiday. All tests were two sided and p-values < 0.05 were considered significant. However, up to 50 biologically plausible associations were tested between 25(OH)D_3 and T<>T and other parameters (e.g. pigmentation, skin type). A p value of <0.001 (0.05/50) is necessary to maintain significance after adjustment for multiple comparisons (Bonferroni correction). Those that do not survive this test are identified in the text. Although these p-values cannot be considered significant, they are indicative of plausible trends that need to be confirmed with a larger sample size.

Results

Personal and ambient UVR exposure and diaries

Mean noon±2h ambient temperature was 19.4^{o}C (range 17.0-25.0). The time outdoors was typically 6-7 hours/day giving a mean exposure of 2.4±1.5(SD) SED (median=2.1) with no differences between skin types I/II and III/IV (p=0.51). Figure 1a shows the accumulation of SED during the holiday. Skin type did not (p=0.43) influence cumulative exposure that was 28.4±4.2 SED in skin types I/II and 29.6±4.1 SED in skin types III/IV. This was also the case when UVR exposure was assessed on days 1-3 (p=0.40), 4-6 (p=0.64), 7-9 (p=0.97) or 9-12 (p=0.17). Days 1–6 were less sunny than days 7–12, so the ambient UVR data in Figure 1b have been split accordingly. Figure 1b also shows the mean SED/0.5h measured on the personal dosimeters. The cumulative exposures were 11.8±2.1 and 11.7±2.3 SED for skin types I/II and III/IV respectively during days 1-6 (~8% of ambient). The comparable data for

days 7-12 were 16.6±3.2 and 17.9±3.5 SED (~9% of ambient). There was no effect of gender on UVR exposure over the whole study period (p=0.72).

The diary results are summarised in table 1. Behaviour is likely to have been influenced by weather (Figure 1b). The children spent time of the beach for 6-8 days with no significant difference between the skin type groups (p = 0.261). Clothing was not recorded (apart from wearing T-shirts) but, when playing on the beach, the children wore hats; the boys wore shorts and the girls wore bikinis. The children were not able to apply sunscreen to their backs but used T-shirts to protect shoulders and back, with at least 56% using this strategy (table 1 – shoulders exposed) when playing on the beach. Sunscreens were used on 200/384 person/days (52%). The most common SPF was 30 (46.5% of the sunscreen/person days), with lower SPF (6-25) and higher SPF (35-50) on 28.0% and 25.5% of the person/days respectively with no significant difference, within the above SPF groupings, with skin type (p=0.410). Overall, there were no skin type dependent significant differences in sun exposure behaviour. However, skin types I/II had significantly more days with sunburn on any body site, than skin types III/IV (p=0.039), but this difference was not significant for specific body sites.

Skin reflectance

There was more pigmentation in skin types III/IV than I/II on all exposed body sites. This was significant for the back (p=0.012), forehead (p=0.0078) and hand (p=0.00049) in June, and for the forehead in July (p=0.015). The holiday significantly (p<0.03) increased pigmentation on all these sites in all skin types, but this increase was not significantly different between skin types I/II and III/IV (p >0.14). However, there was a significant loss of pigmentation in all skin types (p=0.005) on the upper buttock with a significantly (p = 0.02) greater the loss in skin types III/IV compared with I/II. Skin redness was generally higher in skin types I/II than

III/IV, but this was only significant (p=0.038) for the hand in June. There was no significant increase in skin redness for any body site over the holiday (p>0.09).

25(OH)D_3, PTH, crosslaps and osteocalcin

Figure 2a shows the individual pre- and post-holiday 25(OH)D_3 values. Five/32 children (16%) were insufficient in June (<50 nmol/L 25(OH)D_3). The average increase (64.7±13.3 (median=64.9) → 79.3±18.7 (median=79.8) nmol/L) was highly significant ($p=1.59x10^{-7}$), with Δ=14.7±12.4 nmol/L 25(OH)D_3. The mean fold increase was 1.24±0.19. There was no significant (p=0.626) correlation between baseline June 25(OH)D_3 and post-holiday response, i.e. Δ25(OH)D_3.

The boys had more 25(OH)D_3 than girls in June (73.98 vs. 60.41 nmol/L, p=0.0055 but significance lost after Bonferroni correction (SLABC)) and July (93.1 vs. 73.1 nmol/L, p=0.0032 (SLABC)), but there was no influence of gender or skin type (p≥0.17) on June-July Δ 25(OH)D_3. There was no relationship between total cumulative UVR exposure and the July 25(OH)D_3 levels (p=0.388) and for the Δ 25(OH)D_3 (p=0.741). However, there was a borderline association between total UVR exposure and Δ 25(OH)D_3 for skin types III/IV only (p=0.0502). The lack of an overall UVR dose effect is also supported by table 2, which shows a higher Δ 25(OH)D_3 value in the high vs. low SED exposure group but the difference did not reach significance. There were inverse relationships between July 25(OH)D_3 and pigmentation of the outer arm in June (r=-0.468, p=0.008 (SLABC)) and July (r=-0.396, p=0.025 (SLABC)), and Δ pigmentation of the forehead (r=-0.352, p=0.048 (SLABC)).

Figure 2b shows October post-summer (n=29/32) 25(OH)D_3 compared with post-holiday. In most cases (76% children) the values fell (mean of 79.3±18.7→68.2±13.7 nmol/L) and the

average decline (7.6±10.1 nmol/L) is highly significant ($p=3.55x10^{-4}$) for the 29 children who could be compared, two of whom (7%) had 25(OH)D_3 measurements <50 nmol/L. The July ($R^2=0.56$, $p=7.6x10^{-7}$) and October ($R^2=0.51$, $p=1.46x10^{-5}$) 25(OH)D_3 levels were predicted by the June level, and the October level ($R^2=0.52$, $p=1.02x10^{-5}$) was predicted by the July level, with no influence of gender, skin type or total UVR exposure in any case. There was no gender effect on October 25(OH)D_3 (boys=72.2 nmol/L and girls=66.7 nmol/L, p=0.34).

There were no significant differences between pre- and post-holiday (p>0.13) PTH (34.6±13.8 (SD) vs. 37.6±16.7 pg/mL (Δ=3.0±11.1)), crosslaps (1.9±0.5 vs. 2.0±0.5 ng/mL (Δ=0.1±0.4)) and osteocalcin (60.3±12.6 vs. 63.3±11.6 ng/mL (Δ=3.1±11.4)). There was a positive association between Δ crosslaps and Δ osteocalcin ($p = 0.008$, $R^2 = 0.21$) but no other associations between differences in 25(OH)D_3, PTH and the markers of bone turnover ($p \geq 0.060$). However, the boys had a significant (p=0.016) fall of post-holiday osteocalcin compared with the girls (table 2).

DNA damage (T<>T)

There was a highly significant ($p=2.66x10^{-11}$) increase in urinary T<>T (fmol/μmol creatinine) after the holiday (note that data from 4/32 July samples were not used because they were considered unreliable due to low creatinine levels). The mean value increased from 26.9±17.9 (median=22.4) fmol/μmol creatinine (range: 8.0-83.0) to 248.9±113.4 (median=220.9) fmol/μmol creatinine (range: 24.9-522.5). This represents a mean fold increase of 12.62±10.0. Pre- and post-holiday values are shown in figure 3a. June T<>T was not predictive for July (p=0.114). There was no relationship between total cumulative SED and T<>T in July (p=0.892), or between DNA damage and SED over any of the 4 sub-periods (p=0.093-0.934).

Figure 3b shows that skin type had no influence (p=0.945) on the June T<>T measurements (n=32) (mean=27.1±17.6 and 26.7±18.8 fmol/μmol creatinine for I/II and III/IV respectively with corresponding medians of 21.5 and 23.6). Figure 3c shows that there was a borderline significant skin type dependent difference (p=0.0496 (SLABC)) in July T<>T (n=28) (mean=287.7±100.0 and 204.1±114.9 fmol/μmol creatinine for I/II and III/IV respectively with corresponding medians of 305.0 and 184.4), which was independent of the June values. However, there was no skin type dependent difference (p=0.057) for Δ T<>T (table 2).

There was a significant correlation for all skin types between pre-holiday pigmentation on the forehead and June T<>T (p=0.041(SLABC), r=0.364), but no other pre-holiday correlations with the reflectance data (p > 0.279). There was a significant correlation between Δ pigmentation on the back of all skin types and July T<>T (p = 0.009 (SLABC), r = 0.484) and Δ T<>T (p=0.020 (SLABC), r=0.438). This was also the case for buttock Δ pigmentation (p = 0.010 (SLABC), r = 0.477 and p=0.010 (SLABC), r=0.478 respectively). There were no other correlations between July T<>T and Δ T<>T with any of the reflectance data (p >0.071).

Relationships between T<>T and 25(OH)D_3

There was no association between July T<>T and 25(OH)D_3 with p=0.151 for Δ (July–June) 25(OH)D_3 and p=0.247 for July 25(OH)D_3. We did not use Δ (July–June) T<>T in these analyses because June T<>T represent DNA repair damage before the holiday, all of which would have been cleared during the holiday. Furthermore, there was no relationship between June and July T<>T (see figure 3a).

Discussion

Daily UVR exposure was 2.4 SED±1.5 (median=2.1) for all skin types which is borderline erythemal on non-acclimatized adult skin types I/II [35]. In a study of 12 children (8-10 years) on holiday in Denmark (~56^{o}N) over the same period in 2009, the mean daily dose was 3.4 SED (median=3.1, range 1.4–7.0 SED), with mean time outdoors of 5.9 hours (median=6.5, range 2.4–8.8 h) (data from a larger study by personal communication [36]). These results are similar to our data, and to those for children in summer in Sweden [23].

The children received 8-9% of ambient erythemal UVR, which is little higher than the 6-7% reported for children of comparable age at 2-day summer schools in Valencia, Spain, while other studies show summer mean exposures ranging from 2.8% to 6.4% of ambient [37]. The Polish children had a cumulative exposure of about 30 SED over 12 days, which represents ~20% of annual burden, based on a study of Danish children [3]. Comparisons are approximations because exposure depends on body site [38].

The diary data (table 1) show no significant skin type differences in behaviour, sunscreen use and sunburn (except for any body site), although indicators of sunburn were generally higher in skin types I/II. The children self-applied sunscreen, and given that adults typically apply much less than necessary to achieve the labelled SPF [39], it is likely that this was also happened with the children. The reflectance data show no significant increase of skin redness, though values in skin types I/II were typically higher than types III/IV. However, there was a significant increase in pigmentation on exposed body sites, but a decrease in pigmentation in the upper buttock, especially in skin types III/IV. The reason for this is unknown, but this site

is the tan-line region and it is possible that it was more modestly covered in a group holiday than in previous family holidays.

Regular sub-erythemal exposure over small areas is advocated for maintaining optimal vitamin D status in adults [40] , and at least 50 nmol/L (20 ng/mL) is recommended in children for bone health [10,12,41]. One study on fully clothed (without hats) exclusively breastfed babies showed that 2h sunshine/week were needed to maintain $25(OH)D_3$ >27.5 nmol/L, compared to 0.5h/week for those wearing only a diaper/nappy [42]. However, a recent global consensus on rickets prevention was unable to recommend a safe level UVR exposure to enhance vitamin D status [43].

Five (16%) children were insufficient in June but none in July. It should be noted that there is no vitamin D food fortification in Poland. There was a modest (x1.24±0.19) but highly significant increase (Δ=14.7±12.4 nmol/L) in $25(OH)D_3$ during the holiday (figure 2a) that was not correlated with UVR dose. Overall, this increase was equivalent to ~0.5 nmol/L $25(OH)D_3$ per SED (excluding any sunscreen effect). The lack of a baseline $25(OH)D_3$ effect in the children suggests that the increase in $25(OH)D_3$ is likely to have been limited by the relatively high June $25(OH)D_3$ values, because laboratory and holiday studies in adults have shown increased $25(OH)D_3$ in response to UVR is inversely related to its baseline [34,44]. June $25(OH)D_3$ measurements predicted those for July and October, which might indicate individual behavioural trends and/or genetic/metabolic parameters.

The clinical significance of any increase in $25(OH)D_3$ depends its baseline level. The important factor is to achieve sufficiency. Thus, the relatively modest increase will have been more important in the children who were insufficient at the start of the holiday. However, it is

also important to build up summer reserves to maintain sufficiency in winter. We lack data on the half-life of solar UVR-induced $25(OH)D_3$ but a recent study in adults suggests that this is inversely related to the level attained when laboratory UVR intervention was stopped [45].

The study was insufficiently powered to detect skin type effects, but table 2 shows a trend for a greater increase of $25(OH)D_3$ in skin types I/II. A 4-week outdoor study on children in India showed a significantly greater increase in $25(OH)D_3$ in those with light (type IV) compared to dark brown (type V) skin [46]. A review of studies on the role of skin type on vitamin D synthesis reported contradictory results [47].

Adult studies have shown that males have more serum $25(OH)D_3$ than females [48,49]. The boys had higher $25(OH)D_3$ than girls in June and July (but significance was lost after correction for multiple comparisons), with no significant gender effect on the Δ $25(OH)D_3$ values, which suggests a comparable ability for $25(OH)D_3$ production because there was no gender difference in total holiday UVR exposure ($p=0.72$). These differences suggest that the boys spent more time outdoors than the girls before June and are consistent with the observation that serum $25(OH)D_3$ was best predicted (in adults) by solar UVR exposure 6 weeks prior to measurement [48]. However, it should be noted that there was a trend (table 2) for a greater response in the boys. The gender difference in $25(OH)D_3$ was lost by October, by which time there was a significant decline of $25(OH)D_3$, with two (7%) children with < 50 nmol/L (insufficient), and two just above this level.

There was no overall effect of UVR exposure and other parameters on markers of bone turnover (osteocalcine, crosslaps) and PTH. The latter was within the expected range (11-54pg/mL) for children, and is in accord with a study that showed no association between PTH

and serum in children (n=271, mean age 10.4 years) when the concentration of $25(OH)D_3$ was >25 nmol/L [50]. The crosslaps values were similar to those of healthy French [51] and Italian [52] children of the same age (using the same assay) and the osteocalcin values were comparable to those of healthy Italian [53] and Danish [54] children of the same age (different assays). One study of 2798 10 year old German children showed that $25(OH)D_3$ had an inverse seasonal relationship with osteocalcin and crosslaps [55]. Our data show a positive correlation between Δ osteocalcin and crosslaps, and that there was a post-holiday significant reduction of osteocalcin in the boys compared to an increase in the girls. The reasons for this are unclear. Bone turnover markers in children are affected by many factors, especially growth, puberty and activity levels [27]. It is quite possible that some of the girls had entered puberty with greater growth velocity, whereas boys typically have a later onset of puberty.

T<>T is the most frequent CPD [56], whereas cytosine containing CPD (C<>C, C<>T) are implicated in p53 [57] and PTCH mutations [18] in skin cancers. However, T<>T are indicative of cytosine containing CPD. There was a strong association between UVR dose and urinary T<>T in a laboratory study [21], and in children and adults sunbathing (after adjustment for BSA exposed) for 1-2 days in Sweden [23]. In contrast, the association between UVR dose and urinary T<>T was weak in a 3-week study of Swedish lifeguards [58] with a mean daily dose of 7.7 SED.

The highly significant increase in post-holiday urinary T<>T (see figure 3a) was not correlated with UVR exposure. A significant relationship between Δ back pigmentation and T<>T was lost after adjustment for multiple comparisons. Melanogenesis is a crude proxy for cumulative UVR exposure and there is considerable evidence that it is triggered by the CPD

[59]. The back represents ~18% of total BSA in children[60] and is the largest zone likely to have been exposed during the holiday.

Laboratory studies show that *ex vivo* skin type I/II is more susceptible to T<>T than skin type III/IV exposed to the same dose of UVB [56] or solar simulated radiation (SSR) *in vivo* [61]. However, a review of the literature shows no consistent trends over the whole skin type range [62]. Our study showed no effect of skin type on June T<>T. In July, skin types I/II had ~40% more T<>T than III/IV, though of borderline significance (lost after correction for multiple comparisons) and not explained by differences in UVR exposure. Possible reasons include protection by melanin and/or better NER in skin types III/IV [62,63].

Adult data from a 7-day skiing holiday in Austria combined with a 7-day beach holiday in Tenerife showed a significant relationship between UVB dose, $25(OH)D_3$ and urinary T<>T, after accounting for BSA exposed [44], as well as a relationship between post-holiday T<>T and $25(OH)D_3$. The lack of such relationships in the longer children's study is a likely consequence of little interpersonal variation of UVR exposure doses, because of supervised activities, and the lack of adjustment for BSA exposed. Furthermore, both endpoints are likely to have been close to saturation at the end of the holiday. Mean $25(OH)D_3$ at 79.3 nmol/L is likely to have been approaching a plateau and urinary T<>T at a steady state [58], where the formation and excretion of T<>T are equivalent. This state was attained after ~5 days in the Swedish lifeguard study described above, with T<>T at ~200-250 fmol/μmol creatinine, which is comparable to the children in July, even though their UVR doses were much lower.

Swedish children (n =12, skin type II/III) showed an increase of T<>T (means) from 106±35 to 258±61 fmol/μmol creatinine after two consecutive days of beach exposure with a mean

daily dose of 3.2±1.1 SED [23]. The final T<>T value was very similar to ours, but the initial value was about 4 times greater, probably because the study was done in late summer. The Polish children's post holiday median T<>T of 220.9 fmol/μmol creatinine was the same as Danish holidaymakers after a week in Tenerife (median=220.2 fmol/μmol creatinine), even though the Danes received much higher daily (mean=9.4±7.0 (SD) with range of 0.8 – 32.2 SED) and cumulative UVR doses (57±24.7 with range 21.0–115.0 SED) [64] than the children. Overall, our data suggest that children are more susceptible to DNA photodamage than adults and/or have more efficient NER.

A laboratory study of skin type II adults exposed to 1.3 SED solar simulated radiation thrice weekly for 6 weeks, over ~35% BSA, showed dose-dependent increase of $25(OH)D_3$ that a reached a plateau at 3 weeks (after 11.75 SED) in which the fold increase (1.55±0.24) was comparable to the children. There were no detectable urinary T<>T at 6 weeks [65]. A complex UVR exposure (time) dependent combination of photochemical reactions limits the production of pre-vitamin D [66] that reaches a maximal level in a relatively short time (~3h), which in turn limits the production of $25(OH)D_3$. Thus, daily exposure beyond that necessary for maximal pre-vitamin D will result in increased DNA damage. Thus daily exposure of 6-7 hours skews the outcome towards risk, as demonstrated in this study.

The study strengths are its relevance to "real life"; showing beneficial and harmful effects in the same children. The timing of the holiday, immediately after the school term, limited baseline confounding factors, especially T<>T. The weakness is the small sample size when the population is subdivided by skin type or gender. The significance of assessments based on these categories was lost after correction for multiple comparisons, although the skin type trends are biologically plausible. The study would have been improved if exposed BSA and

sunscreen application thickness had been measured. Group supervision limited the interpersonal variation of personal UVR exposure, which restricts the establishment of UVR dose relationships.

In conclusion, a temperate latitude beach holiday moderately increased $25(OH)D_3$ (x1.24±0.19) in children but was associated with a much greater increase in DNA damage (x12.62±10.0), which was more evident in skin types I/II. Any comparison of risks and benefits should note that brief daily UVR exposures are the safest and most effective way to improve vitamin D status. Prolonged exposure has limited benefit [66], but enhances DNA damage. There is an urgent need for a better understanding of the consequences of childhood UVR exposure and to develop strategies for its optimization.

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References

1 Stern RS, Weinstein MC, Baker SG. Risk reduction for nonmelanoma skin cancer with childhood sunscreen use. *Archives of dermatology* 1986; **122**: 537-45.

2 Stern RS. Proportion of lifetime UV dose received by age 18, what Stern et al actually said in 1986. *The Journal of investigative dermatology* 2005; **124**: 1079-80.

3 Thieden E, Philipsen PA, Sandby-Moller J *et al.* Proportion of lifetime UV dose received by children, teenagers and adults based on time-stamped personal dosimetry. *The Journal of investigative dermatology* 2004; **123**: 1147-50.

4 Gandini S, Autier P, Boniol M. Reviews on sun exposure and artificial light and melanoma. *Progress in biophysics and molecular biology* 2011; **107**: 362-6.

5 Green AC, Wallingford SC, McBride P. Childhood exposure to ultraviolet radiation and harmful skin effects: epidemiological evidence. *Progress in biophysics and molecular biology* 2011; **107**: 349-55.

6 Corona R, Dogliotti E, D'Errico M *et al.* Risk factors for basal cell carcinoma in a Mediterranean population: role of recreational sun exposure early in life. *Archives of dermatology* 2001; **137**: 1162-8.

7 Wong JR, Harris JK, Rodriguez-Galindo C *et al.* Incidence of childhood and adolescent melanoma in the United States: 1973-2009. *Pediatrics* 2013; **131**: 846-54.

8 Skellett AM, Hafiji J, Greenberg DC *et al.* The incidence of basal cell carcinoma in the under-30s in the UK. *Clinical and experimental dermatology* 2012; **37**: 227-9.

9 Macdonald HM, Mavroeidi A, Fraser WD *et al.* Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2011; **22**: 2461-72.

10 Braegger C, Campoy C, Colomb V *et al.* Vitamin D in the healthy European paediatric population. *Journal of pediatric gastroenterology and nutrition* 2013; **56**: 692-701.

11 Absoud M, Cummins C, Lim MJ *et al.* Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One* 2011; **6**: e22179.

12 Holick MF. The D-lightful vitamin D for child health. *JPEN. Journal of parenteral and enteral nutrition* 2012; **36**: 9S-19S.

13 Elder CJ, Bishop NJ. Rickets. *Lancet* 2014.

14 Goldacre M, Hall N, Yeates DG. Hospitalisation for children with rickets in England: a historical perspective. *Lancet* 2014; **383**: 597-8.

15 Abrams SA, Coss-Bu JA, Tiosano D. Vitamin D: effects on childhood health and disease. *Nature reviews. Endocrinology* 2013; **9**: 162-70.

16 Autier P, Boniol M, Pizot C *et al.* Vitamin D status and ill health: a systematic review. *The lancet. Diabetes & endocrinology* 2014; **2**: 76-89.

17 Young AR, Chadwick CA, Harrison GI *et al.* The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *The Journal of investigative dermatology* 1998; **111**: 982-8.

18 Jayaraman SS, Rayhan DJ, Hazany S *et al.* Mutational landscape of basal cell carcinomas by whole-exome sequencing. *The Journal of investigative dermatology* 2014; **134**: 213-20.

19 Kripke ML, Cox PA, Alas LG *et al.* Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proceedings of the National Academy of Sciences of the United States of America* 1992; **89**: 7516-20.

20 Dutton-Regester K, Kakavand H, Aoude LG *et al.* Melanomas of unknown primary have a mutation profile consistent with cutaneous sun-exposed melanoma. *Pigment cell & melanoma research* 2013; **26**: 852-60.

21 Kotova N, Hemminki K, Segerback D. Urinary thymidine dimer as a marker of total body burden of UV-inflicted DNA damage in humans. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2005; **14**: 2868-72.

22 Cooke MS, Harry EL, Liljendahl TS *et al.* DNA nucleotide excision repair, where do all the cyclobutane pyrimidine dimers go? *Cell cycle* 2013; **12**: 1642.

23 Liljendahl TS, Kotova N, Segerback D. Quantification of ultraviolet radiation-induced DNA damage in the urine of Swedish adults and children following exposure to sunlight. *Biomarkers* 2012; **17**: 634-41.

24 Young AR, Orchard GE, Harrison GI *et al.* The detrimental effects of daily sub-erythemal exposure on human skin in vivo can be prevented by a daily-care broad-spectrum sunscreen. *The Journal of investigative dermatology* 2007; **127**: 975-8.

25 Rhodes LE, Webb AR, Fraser HI *et al.* Recommended summer sunlight exposure levels can produce sufficient (> or =20 ng ml(-1)) but not the proposed optimal (> or =32 ng ml(-1)) 25(OH)D levels at UK latitudes. *The Journal of investigative dermatology* 2010; **130**: 1411-8.

26 Bogh MK, Schmedes AV, Philipsen PA *et al.* A small suberythemal ultraviolet B dose every second week is sufficient to maintain summer vitamin D levels: a randomized controlled trial. *The British journal of dermatology* 2012; **166**: 430-3.

27 Szulc P, Seeman E, Delmas PD. Biochemical measurements of bone turnover in children and adolescents. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2000; **11**: 281-94.

28 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Archives of dermatology* 1988; **124**: 869-71.

29 Thieden E, Philipsen PA, Wulf HC. Compliance and data reliability in sun exposure studies with diaries and personal, electronic UV dosimeters. *Photodermatology, photoimmunology & photomedicine* 2006; **22**: 93-9.

30 Bodekaer Larsen M, Petersen B, Philipsen PA *et al.* Sun exposure and protection behaviour of Danish farm children Parental influence on their children. *Photochemistry and photobiology* 2014.

31 Thieden E, Philipsen PA, Heydenreich J *et al.* UV radiation exposure related to age, sex, occupation, and sun behavior based on time-stamped personal dosimeter readings. *Archives of dermatology* 2004; **140**: 197-203.

32 Datta P, Philipsen PA, Olsen P *et al.* Major inter-personal variation in the increase and maximal level of 25-hydroxy vitamin D induced by UVB. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2016; **15**: 536-45.

33 Seaton B, Ali A. Simplified manual high performance clinical chemistry methods for developing countries. *Med. Lab. Sci.* 1984; **41**: 327-36.

34 Bogh MK, Schmedes AV, Philipsen PA *et al.* Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. *The Journal of investigative dermatology* 2010; **130**: 546-53.

35 Harrison GI, Young AR. Ultraviolet radiation-induced erythema in human skin. *Methods* 2002; **28**: 14-9.

36 Bodekaer M, Petersen B, Thieden E *et al.* UVR exposure and vitamin D in a rural population. A study of outdoor working farmers, their spouses and children. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2014; **13**: 1598-606.

37 Serrano MA, Canada J, Moreno JC *et al.* Occupational UV exposure of environmental agents in Valencia, Spain. *Photochemistry and photobiology* 2014; **90**: 911-8.

38 Thieden E, Agren MS, Wulf HC. The wrist is a reliable body site for personal dosimetry of ultraviolet radiation. *Photodermatology, photoimmunology & photomedicine* 2000; **16**: 57-61.
39 Petersen B, Datta P, Philipsen PA *et al.* Sunscreen use and failures--on site observations on a sun-holiday. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2013; **12**: 190-6.
40 Working Group of the A, New Zealand B, Mineral S *et al.* Vitamin D and adult bone health in Australia and New Zealand: a position statement. *The Medical journal of Australia* 2005; **182**: 281-5.
41 Winzenberg T, Powell S, Shaw KA *et al.* Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. *Bmj* 2011; **342**: c7254.
42 Specker BL, Valanis B, Hertzberg V *et al.* Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infants. *The Journal of pediatrics* 1985; **107**: 372-6.
43 Munns CF, Shaw N, Kiely M *et al.* Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. *The Journal of clinical endocrinology and metabolism* 2016; **101**: 394-415.
44 Petersen B, Wulf HC, Triguero-Mas M *et al.* Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage. *The Journal of investigative dermatology* 2014; **134**: 2806-13.
45 Datta P, Philipsen PA, Olsen P *et al.* The half-life of 25(OH)D after UVB exposure depends on gender and vitamin D receptor polymorphism but mainly on the start level. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2017; **16**: 985-95.
46 Marwaha RK, Sreenivas V, Talwar D *et al.* Impact of solar ultraviolet B radiation (290-320 nm) on vitamin D synthesis in children with type IV and V skin. *The British journal of dermatology* 2015; **173**: 604-6.
47 Xiang F, Lucas R, de Gruijl F *et al.* A systematic review of the influence of skin pigmentation on changes in the concentrations of vitamin D and 25-hydroxyvitamin D in plasma/serum following experimental UV irradiation. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2015; **14**: 2138-46.
48 Nair-Shalliker V, Clements M, Fenech M *et al.* Personal sun exposure and serum 25-hydroxy vitamin D concentrations. *Photochemistry and photobiology* 2013; **89**: 208-14.
49 Webb AR, Kift R, Durkin MT *et al.* The role of sunlight exposure in determining the vitamin D status of the U.K. white adult population. *The British journal of dermatology* 2010; **163**: 1050-5.
50 Crews BO, Moore J, Dietzen DJ. Circulating intact parathyroid hormone is suppressed at 25-hydroxyvitamin D concentrations >25 nmol/L in children. *Journal of pediatric endocrinology & metabolism : JPEM* 2014; **27**: 657-60.
51 Alberti C, Chevenne D, Mercat I *et al.* Serum concentrations of insulin-like growth factor (IGF)-1 and IGF binding protein-3 (IGFBP-3), IGF-1/IGFBP-3 ratio, and markers of bone turnover: reference values for French children and adolescents and z-score comparability with other references. *Clinical chemistry* 2011; **57**: 1424-35.
52 Gennai I, Di Iorgi N, Reggiardo G *et al.* Age- and sex-matched reference curves for serum collagen type I C-telopeptides and bone alkaline phosphatase in children and adolescents: An alternative multivariate statistical analysis approach. *Clin Biochem* 2016; **49**: 802-7.
53 Cioffi M, Molinari AM, Gazzerro P *et al.* Serum osteocalcin in 1634 healthy children. *Clinical chemistry* 1997; **43**: 543-5.
54 Schou AJ, Heuck C, Wolthers OD. Vitamin D supplementation to healthy children does not affect serum osteocalcin or markers of type I collagen turnover. *Acta Paediatr* 2003; **92**: 797-801.

55 Thiering E, Bruske I, Kratzsch J *et al.* Associations between serum 25-hydroxyvitamin D and bone turnover markers in a population based sample of German children. *Scientific reports* 2015; **5**: 18138.
56 Douki T. The variety of UV-induced pyrimidine dimeric photoproducts in DNA as shown by chromatographic quantification methods. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2013; **12**: 1286-302.
57 Giglia-Mari G, Sarasin A. TP53 mutations in human skin cancers. *Human mutation* 2003; **21**: 217-28.
58 Liljendahl TS, Blomqvist A, Andersson EM *et al.* Urinary levels of thymine dimer as a biomarker of exposure to ultraviolet radiation in humans during outdoor activities in the summer. *Mutagenesis* 2013; **28**: 249-56.
59 Agar N, Young AR. Melanogenesis: a photoprotective response to DNA damage? *Mutation research* 2005; **571**: 121-32.
60 Hettiaratchy S, Papini R. Initial management of a major burn: II--assessment and resuscitation. *Bmj* 2004; **329**: 101-3.
61 Xu G, Snellman E, Bykov VJ *et al.* Effect of age on the formation and repair of UV photoproducts in human skin in situ. *Mutation research* 2000; **459**: 195-202.
62 Fajuyigbe D, Young AR. The Impact of Skin Colour on Human Photobiological Responses. *Pigment cell & melanoma research* 2016.
63 Sheehan JM, Cragg N, Chadwick CA *et al.* Repeated ultraviolet exposure affords the same protection against DNA photodamage and erythema in human skin types II and IV but is associated with faster DNA repair in skin type IV. *The Journal of investigative dermatology* 2002; **118**: 825-9.
64 Petersen B, Thieden E, Philipsen PA *et al.* A sun holiday is a sunburn holiday. *Photodermatology, photoimmunology & photomedicine* 2013; **29**: 221-4.
65 Felton SJ, Cooke MS, Kift R *et al.* Concurrent beneficial (vitamin D production) and hazardous (cutaneous DNA damage) impact of repeated low-level summer sunlight exposures. *The British journal of dermatology* 2016.
66 Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *The Journal of clinical endocrinology and metabolism* 1988; **67**: 373-8.

Event	Qualifier	Skin type			p for I/II vs III/IV	Test used
		n = children (%)				
		All (n = 32)	I/II[4] (n = 18)	III/IV[5] (n = 14)		
No of days[1] Sunbathing[2]	4 x	11 (34%)	5 (28%)	6 (43%)	0.718	Chi[2]
	5 x	16 (50%)	10 (56%)	6 (43%)		
	6 x	5 (16%)	3 (17%)	2 (14%)		
	7 x	25 (78%)	16 (89%)	9 (64%)		
	8 x	1 (3%)	0 (0%)	1 (7%)		
No of days[1]with shoulders exposed	2 x	1 (3%)	0 (0%)	1 (7%)	0.966	Chi[2]
	3 x	5 (16%)	3 (17%)	2 (14%)		
	4 x	11 (34%)	6 (33%)	5 (36%)		
	5 x	13 (41%)	8 (44%)	5 (36%)		
	6 x	2 (6%)	1 (6%)	1 (7%)		
Sunscreen application days (max =12)	**Body site**	**n = mean days (SD) sunscreen applied**				
	Face	5.5 (2.2)	5.8 (2.7)	5.1 (1.4)	0.396	T
	Shoulders	3.6 (2.4)	4.2 (2.8)	2.9 (1.6)	0.154	T
	Arms	5.8 (1.9)	5.8 (2.4)	5.8 (0.8)	0.944	T
	Chest	3.3 (2.3)	3.7 (2.7)	2.8 (1.8)	0.264	T
	Legs	5.2 (1.9)	5.5 (2.3)	4.8 (1.4)	0.305	T
Sunburn	**Occurrence**	**n = children (%)**				
	≥Once on any body site	26 (81%)	16 (89%)	10 (71%)	0.365	Chi[2]
	Persistent next day on a given body site[3]	17 (65%)	11 (69%)	6 (60%)	0.476	Chi[2]
Sunburned days (max = 12)	**Body site**	**n = mean days with sunburn**				
	Face	1.0	1.4	0.5	0.189	M-W
	Shoulders	1.3	1.7	0.6	0.084	M-W
	Arms	1.4	1.6	1.2	0.343	M-W
	Chest	0.4	0.4	0.3	0.830	M-W
	Legs	0.03	0.1	0	1.000	M-W
	Any site	1.8	2.2	1.2	0.039	T

Table 1 Summary of data from sun diaries during the summer camp. The back was not assessed because the children could not self-apply sunscreen. A day with shoulders exposed is indicative of not wearing a T-shirt.
[1]A maximum of 12 days was possible but behaviour was influenced by weather (See Fig 1b). [2]Sunbathing is defined as intentional sun exposure with the upper body exposed. [3]The participants reported that the sunburn was present for at least two consecutive days on the same body site, which is indicative of more severe sunburn that did not resolve within a day. [4]Of 18 skin types I/II, 2 were skin type I. [5]Of 14 skin types III/IV, 2 were skin type IV. M-W = Mann-Whitney, T = unpaired t-test

Category		**N**	**Mean total SED**	**Mean Δ T<>T (fmol/µmol creatinine)**	**Mean Δ 25(OH)D_3 (nmol/L)**	**Mean Δ PTH (pg/mL)**	**Mean Δ osteocalcin (ng/mL)**	**Mean Δ crosslaps (ng/mL)**
All		32	28.9 ± 4.1	223.9 ± 109.7	14.7±12.4	3.0 ± 11.1	3.1 ± 11.4	0.1 ± 0.4
Skin type	I/II	18	28.4 ± 4.2	260.5 ± 97.7	17.3 ± 15.6	2.5 ± 12.3	2.2 ± 13.6	0.2 ± 0.5
	III/IV	14	29.6 ± 4.1	181.7 ± 111.1	11.3 ± 5.1	3.5 ± 9.7	4.1 ± 8.1	0.0 ± 0.2
	p-value		0.428	0.057	0.182	0.806	0.640	0.443
Gender	F	22	29.1 ± 4.2	226.0 ± 115.1	12.7 ± 10.1	5.5 ± 11.3	6.2 ± 12.0	0.2 ± 0.5
	M	10	28.5 ± 4.2	218.6 ± 102.1	19.1 ± 15.9	-2.5 ± 8.8	-4.0 ± 5.7	-0.1 ± 0.3
	p-value		0.720	0.874	0.174	0.059	0.016	0.178
UVR exposure	Low SED	17	25.7 ± 1.8	222.4 ± 89.7	12.8 ± 12.7	4.2 ± 11.2	4.6 ± 9.4	0.2 ± 0.5
	High SED	15	32.6 ± 2.6	225.4 ± 130.2	16.8 ± 12.0	1.6 ± 11.1	1.3 ± 13.4	0.0 ± 0.3
	p-value		6.102×10^{-10}	0.943	0.375	0.509	0.414	0.312

Table 2 Total holiday exposure and impact on DNA damage (T<>T), 25(OH)D_3 and markers of bone turnover (±SD). Low SED are values below the mean (n = 32) and high SED above the mean. All p-values are from unpaired T-test

Figure Legends

1. (a) Children's mean cumulative SED (b) Mean daily ambient SED/0.5h and corresponding values from the personal dosimeters. The data are split into days 1 - 6 and 7 - 12 because of their different weather patterns.
2. (a) Pre- and post-holiday 25(OH)D_3 values (n = 32) with mean increase of 64.7 ± 13.3 → 79.3 ± 18.7 (SD) nmol/L (b) Post-holiday and October 25(OH)D_3 values with mean fall of 79.3 ± 18.7 → 68.2 ± 13.7 (SD) nmol/L. Note: October data missing for 3 children. Note: dotted lines on both figures show the 50nmol/L threshold for sufficiency.
3. (a) Comparison of DNA damage before and after holiday sun exposure (n = 28) with mean T<>T increase of 25.15 fmol/μmol (range: 7.97-68.64) to 248.9 fmol/μmol (range: 24.91-522.50). Note: 10-fold difference in scale. (b) There was no skin type dependent difference in T<>T on entry to the study (p = 0.945) but (c) Skin types I/II had more DNA damage than skin types III/IV at the end of the study (p = 0.0496 – significance lost after Bonferroni correction). Note: 4 samples taken in July not used because of low creatinine values.

Figure 1a

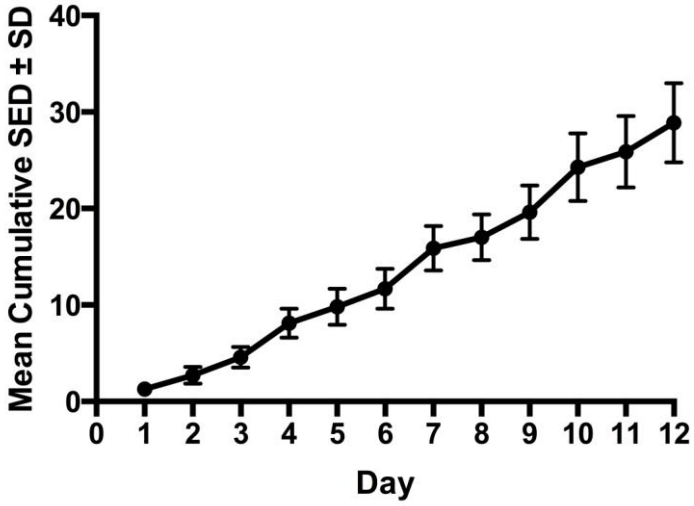
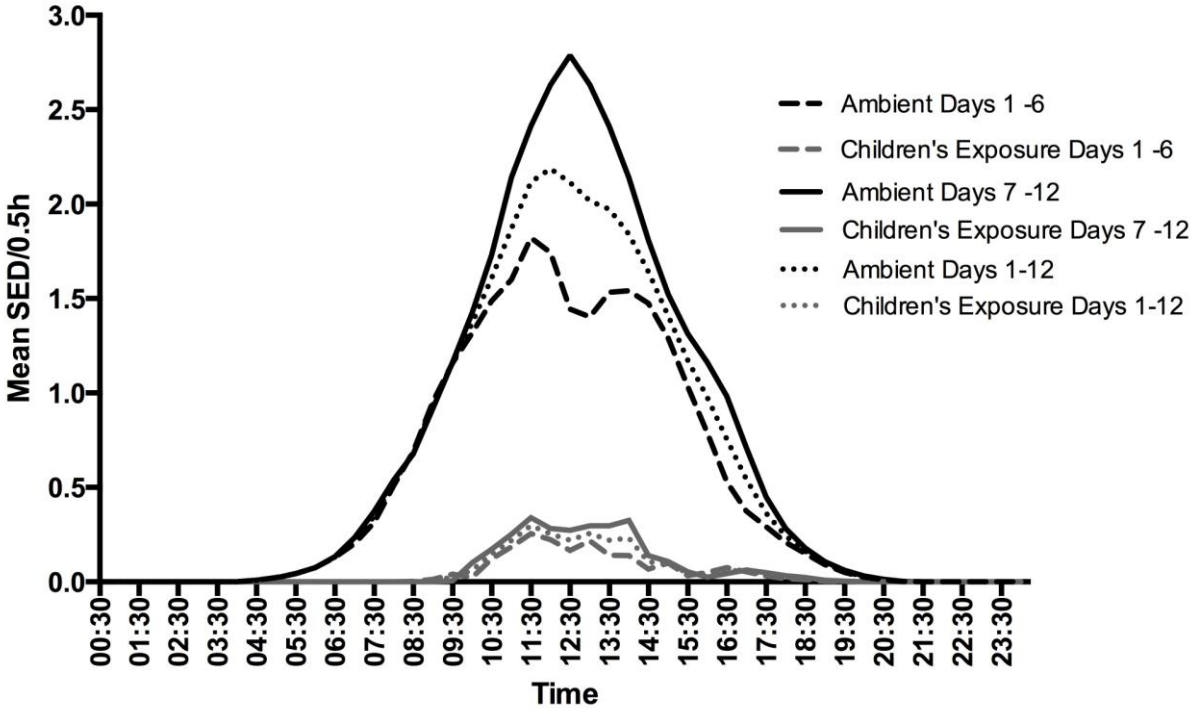
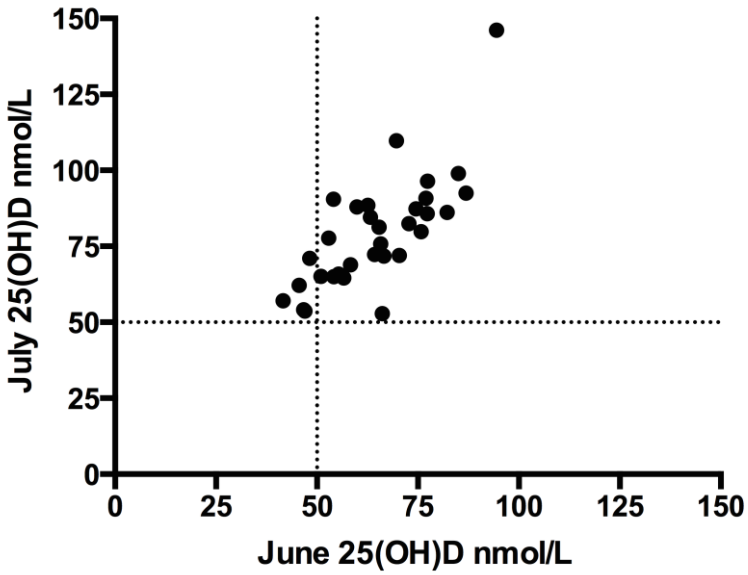
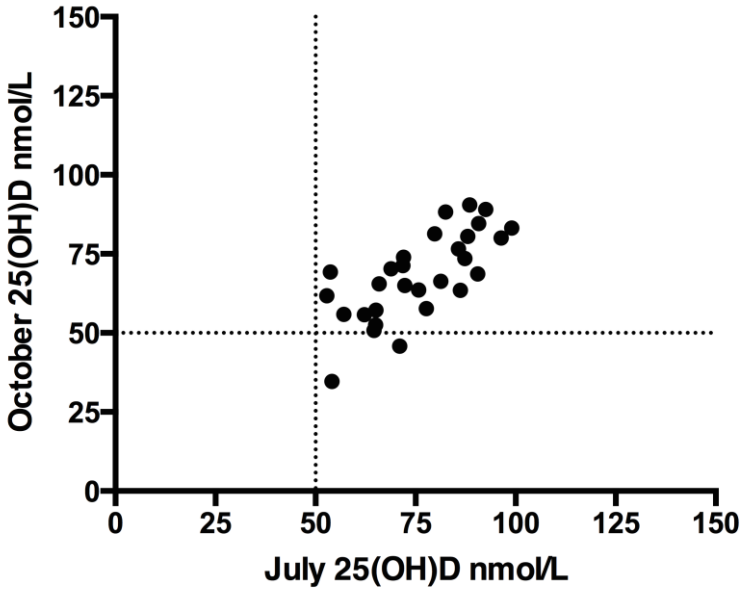


Figure 1b







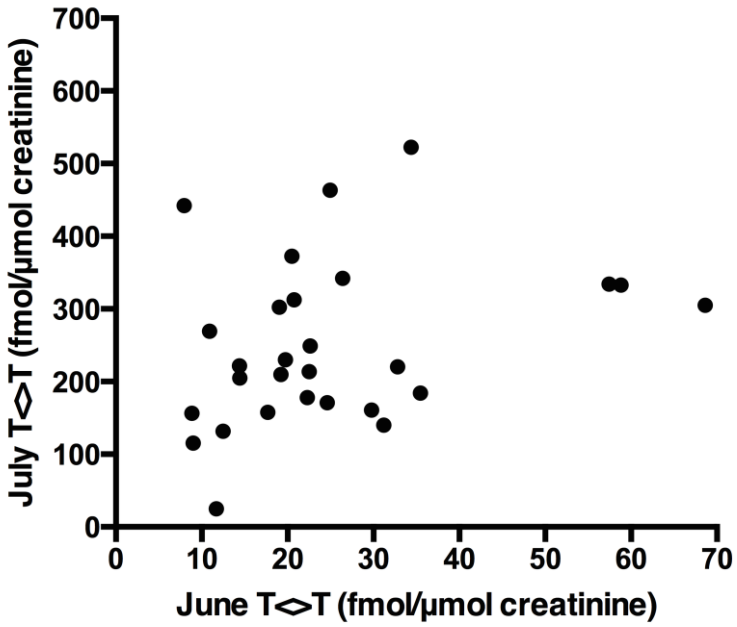


Figure 3b

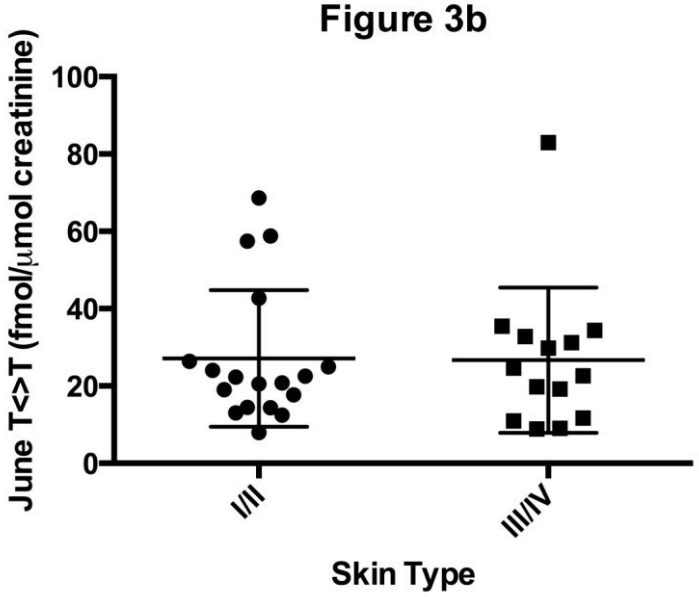


Figure 3c